

09/765555

(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, CABA, AGRICOLA, FSTA, CROPU, CROPB' ENTERED AT 10:34:50 ON 27 MAR 2003)

L12 118 SEA ABB=ON PLU=ON "BARBAS III C"?/AU  
L13 41 SEA ABB=ON PLU=ON "STEGE J"?/AU  
L14 2369 SEA ABB=ON PLU=ON "GUAN X"?/AU  
L15 1078 SEA ABB=ON PLU=ON "BARBAS C"?/AU  
L16 14 SEA ABB=ON PLU=ON (L12 OR L15) AND L13 AND L14  
L17 15 SEA ABB=ON PLU=ON (L12 OR L15) AND (L13 OR L14)  
L18 14 SEA ABB=ON PLU=ON L13 AND L14  
L19 87 SEA ABB=ON PLU=ON (L12 OR L15 OR L13 OR L14) AND (ZFP?  
OR (ZF OR (ZN OR ZINC)(W) FINGER)(W) PROTEIN)  
L20 35 SEA ABB=ON PLU=ON L19 AND (PLANT OR MAIZE OR CORN OR  
CARROT OR TOBACCO OR TOMATO OR POTATO OR BANANA OR  
SOYABEAN OR SOYBEAN OR (SOY OR SOYA)(W) BEAN OR PEPPER  
OR WHEAT OR RYE OR RICE OR SPINACH)  
L21 36 SEA ABB=ON PLU=ON L16 OR L17 OR L18 OR L20  
L22 13 DUP REM L21 (23 DUPLICATES REMOVED)

- Author(s)

L22 ANSWER 1 OF 13 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2003:133983 HCAPLUS

DOCUMENT NUMBER: 138:182057

TITLE: Usage of **zinc finger**  
**proteins** and their fusions with effector  
domains to regulate gene expression and  
metabolic pathways in **plants**

INVENTOR(S): **Barbas, Carlos F.; Stege, Justin**  
**T.; Guan, Xueni; Dalmia, Bipin**

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 84 pp., Cont.-in-part of  
U.S. Ser. No. 620,897.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003037355	A1	20030220	US 2001-765555	20010119
PRIORITY APPLN. INFO.:			US 2000-177468P	P 20000121
			US 2000-620897	A2 20000721

AB The invention relates to the field of **plant** and  
agricultural technol. More specifically, the invention relates to  
the construction of **zinc finger proteins**  
and fusions of said proteins and their use to regulate gene  
expression and metabolic pathways in **plants**.  
**Plant** genes AP3 and MIPS were examd. for suitable zinc  
finger binding sites. The novel engineered **zinc**  
**finger proteins** used in the present methods are  
**ZFPm1, ZFPm2, ZFPm3, ZFPm4** and  
**ZFPap3**. These proteins can be used alone or fused to an  
effector domain. The present methods can be used to modulate gene  
expression in monocot or dicot **plant** cells.

L22 ANSWER 2 OF 13 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2003:229501 HCAPLUS

TITLE: Zinc fingers and a green thumb: manipulating

Searcher : Shears 308-4994

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AUTHOR(S): gene expression in plants  
Segal, David J.; Stege, Justin T.;  
Barbas, Carlos F.  
CORPORATE SOURCE: The Skaggs Institute for Chemical Biology and  
the Department of Molecular Biology, The Scripps  
Research Institute, La Jolla, CA, 92037, USA  
SOURCE: Current Opinion in Plant Biology (2003), 6(2),  
163-168  
CODEN: COPBFZ; ISSN: 1369-5266  
PUBLISHER: Elsevier Science Ltd.  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Artificial transcription factors can be rapidly constructed from  
predefined zinc-finger modules to regulate virtually any gene.  
Stable, heritable up- and downregulation of endogenous genes has  
been demonstrated in transgenic plants. These advances promise new  
approaches for creating functional knockouts and conditional  
overexpression, and for other gene discovery and manipulation  
applications in plants.

L22 ANSWER 3 OF 13 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 1  
ACCESSION NUMBER: 2002:795317 HCAPLUS  
DOCUMENT NUMBER: 138:84370  
TITLE: Heritable endogenous gene regulation in  
plants with designed polydactyl zinc  
finger transcription factors  
AUTHOR(S): Guan, Xuen; Stege, Justin;  
Kim, Myoung; Dahmani, Zina; Fan, Nancy; Heifetz,  
Peter; Barbas, Carlos F., III; Briggs,  
Steven P.  
CORPORATE SOURCE: Torrey Mesa Research Institute, San Diego, CA,  
92121, USA  
SOURCE: Proceedings of the National Academy of Sciences  
of the United States of America (2002), 99(20),  
13296-13301  
CODEN: PNASA6; ISSN: 0027-8424  
PUBLISHER: National Academy of Sciences  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Zinc finger transcription factors (TFsZF) were designed and applied  
to transgene and endogenous gene regulation in stably transformed  
plants. The target of the TFsZF is the Arabidopsis gene  
APETALA3 (AP3), which encodes a transcription factor that detes.  
floral organ identity. A zinc finger  
protein (ZFP) was designed to specifically bind to  
a region upstream of AP3. AP3 transcription was induced by  
transformation of leaf protoplasts with a transformation vector that  
expressed a TFZF consisting of the ZFP fused to the  
tetrameric repeat of herpes simplex VP16's minimal activation  
domain. Histochem. staining of .beta.-glucuronidase (GUS) activity  
in transgenic AP3::GUS reporter plants expressing GUS  
under control of the AP3 promoter was increased dramatically in  
petals when the AP3-specific TFZF activator was cointroduced.  
TFZF-amplified GUS expression signals were also evident in sepal  
tissues of these double-transgenic plants. Floral  
phenotype changes indicative of endogenous AP3 factor coactivation  
were also obsd. The same AP3-specific ZFPAP3 was also  
fused to a human transcriptional repression domain, the mSIN3

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interaction domain, and introduced into either AP3::GUS-expressing **plants** or wild-type Arabidopsis **plants**. Dramatic repression of endogenous AP3 expression in floral tissue resulted when a constitutive promoter was used to drive the expression of this TFZF. These **plants** were also sterile. When a floral tissue-specific promoter from APETALA1 (AP1) gene was used, floral phenotype changes were also obsd., but in contrast the **plants** were fertile. Our results demonstrate that artificial transcriptional factors based on synthetic **zinc finger proteins** are capable of stable and specific regulation of endogenous genes through multiple generations in multicellular organisms.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L22 ANSWER 4 OF 13 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 2  
ACCESSION NUMBER: 2002:795316 HCAPLUS  
DOCUMENT NUMBER: 138:34056  
TITLE: Regulation of transgene expression in  
**plants** with polydactyl zinc finger  
transcription factors  
AUTHOR(S): Ordiz, M. Isabel; Barbas, Carlos F., III  
; Beachy, Roger N.  
CORPORATE SOURCE: Donald Danforth Plant Science Center, St. Louis,  
MO, 63132, USA  
SOURCE: Proceedings of the National Academy of Sciences  
of the United States of America (2002), 99(20),  
13290-13295  
CODEN: PNASA6; ISSN: 0027-8424  
PUBLISHER: National Academy of Sciences  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Designer zinc finger transcription factors (TFsZF) have been developed to control the expression of transgenes and endogenous genes in mammalian cells. Application of TFsZF technol. in **plants** would enable a wide range of both basic and applied studies. In this paper, we report the use of TFsZF to target a defined 18-bp DNA sequence to control gene expression in **plant** cells and in transgenic **plants**. A .beta.-glucuronidase reporter gene was activated by using the designed six-zinc finger protein 2C7 expressed as a fusion with the herpes simplex virus VP16 transcription factor activation domain. Reporter gene expression was activated 5- to 30-fold by using TFsZF in BY-2 protoplasts, whereas expression was increased as much as 450 times in transgenic **tobacco plants**. Use of a phloem-specific promoter to drive expression of the TFsZF resulted in activation of the reporter gene in vascular tissues. Transgenic **tobacco plants** that produce 2C7 transcription factors were phenotypically normal through two generations, suggesting that the factors exerted no adverse effects. This study demonstrates the utility of zinc finger technol. in **plants**, setting the stage for its application in basic and applied agricultural biotechnol.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

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L22 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 3  
ACCESSION NUMBER: 2003:81400 HCAPLUS  
TITLE: Controlling gene expression in **plants**  
using synthetic zinc finger transcription  
factors  
AUTHOR(S): **Stege, Justin T.; Guan, Xuen**  
; Ho, Thao; Beachy, Roger N.; **Barbas,**  
**Carlos F., III**  
CORPORATE SOURCE: Department of Molecular Biology and The Skaggs  
Institute for Chemical Biology, Scripps Research  
Institute, La Jolla, CA, 92037, USA  
SOURCE: Plant Journal (2002), 32(6), 1077-1086  
CODEN: PLJUED; ISSN: 0960-7412  
PUBLISHER: Blackwell Science Ltd.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Synthetic **zinc finger proteins** can be  
fused to transcriptional regulatory domains to create artificial  
transcription factors that modulate the expression of a specific  
target gene. Recent studies have demonstrated that synthetic zinc  
finger domains can be constructed to bind DNA sequences with a high  
degree of specificity. To devise a general strategy for controlling  
**plant** gene expression with artificial transcription factors,  
a rapid transient assay was developed to test the regulatory  
activity of synthetic zinc finger transcription factors (effectors)  
on target plasmids (reporters) in **plant** cells. Effective  
activation was demonstrated with **zinc finger**  
**proteins** fused to a deriv. of the VP16 activation domain.  
The mSin3 interaction domain (SID) of the human MAD1 protein  
provided moderate repression of target reporters. Unlike many  
naturally occurring transcription factors, these synthetic effectors  
exhibit a strong dependence on binding site position. Reporter  
genes that are stably integrated into **plant** cells  
responded similarly to transiently transfected reporter plasmids,  
verifying that this assay accurately reflects the behavior of these  
transcription factors on an endogenous target within the context of  
chromosomal DNA. These results provide evidence that synthetic  
**zinc finger proteins** can be used to  
manipulate the expression of endogenous genes in **plants**.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L22 ANSWER 6 OF 13 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 4  
ACCESSION NUMBER: 2002:123927 HCAPLUS  
DOCUMENT NUMBER: 137:42141  
TITLE: Engineering polydactyl zinc-finger transcription  
factors  
AUTHOR(S): Beerli, Roger R.; **Barbas, Carlos F., III**  
CORPORATE SOURCE: The Skaggs Institute for Chem. Biology and Dep.  
of Molecular Biology, The Scripps Research  
Institute, La Jolla, CA, 92037, USA  
SOURCE: Nature Biotechnology (2002), 20(2), 135-141  
CODEN: NABIF9; ISSN: 1087-0156  
PUBLISHER: Nature America Inc.  
DOCUMENT TYPE: Journal; General Review  
LANGUAGE: English

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AB A review. The availability of rapid and robust methods for controlling gene function is of prime importance not only for assigning functions to newly discovered genes, but also for therapeutic intervention. Traditionally, gene function has been probed by often-laborious methods that either increase the level of a gene product or decrease it. Advances now make it possible to rapidly produce **zinc-finger proteins** capable of recognizing virtually any 18 bp stretch of DNA - a sequence long enough to specify a unique address in any genome. The attachment of functional domains also allows the design of tailor-made transcription factors for specific genes. Recent studies demonstrate that artificial transcription factors are capable of controlling the expression of endogenous genes in their native chromosomal context with a high degree of specificity in both animals and **plants**. Dominant regulatory control of expression of any endogenous gene can be achieved rapidly and can be also placed under chem. control. A wide range of potential applications is now within reach.

REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 7 OF 13 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 5

ACCESSION NUMBER: 2001:545414 HCAPLUS

DOCUMENT NUMBER: 135:133107

TITLE: Usage of **zinc finger protein** to regulate gene expression and metabolic pathways in **plants** and creation of five **zinc finger proteins**

INVENTOR(S): **Barbas, Carlos F., III; Stege, Justin T.; Guan, Xue Ni; Dalmia, Bipin**

PATENT ASSIGNEE(S): Scripps Research Institute, USA

SOURCE: PCT Int. Appl., 156 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001052620	A2	20010726	WO 2001-US1817	20010119
WO 2001052620	A3	20020207		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 2001029641	A5	20010731	AU 2001-29641	20010119
EP 1276869	A2	20030122	EP 2001-942508	20010119
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR			

PRIORITY APPLN. INFO.: US 2000-177468P P 20000121  
US 2000-620897 A 20000721

Searcher : Shears 308-4994

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WO 2001-US1817 W 20010119

AB The invention relates to the field of **plant** and agricultural technol. More specifically, the invention relates to the use of **zinc finger proteins** and fusions of said proteins to regulate gene expression and metabolic pathways in **plants**. The genes, AP3 and MIPS, were examd. for suitable zinc finger binding sites. Five new **zinc finger proteins, ZFPap3, ZFPm1, ZFPm2, ZFPm3 and ZFPm4**, were constructed from human **zinc finger protein Sp1C**, expressed in *E. coli* and purified. DNA binding specificity of **ZFPap3, ZFPm1, ZFPm2, ZFPm3 and ZFPm4** was characterized.

L22 ANSWER 8 OF 13 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:408061 HCAPLUS

DOCUMENT NUMBER: 135:30537

TITLE: Design, construction and of **zinc**

**finger protein** derivatives and their use in the modulation of gene expression

INVENTOR(S): **Barbas, Carlos F., III**; Gottesfeld, Joel M.; Wright, Peter E.

PATENT ASSIGNEE(S): The Scripps Research Institute, USA

SOURCE: U.S., 67 pp., Cont.-in-part of U.S. Ser. No. 312,604, abandoned.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6242568	B1	20010605	US 1996-676318	19961230
WO 9519431	A1	19950720	WO 1995-US829	19950118
W: AU, CA, FI, JP, NO, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				

PRIORITY APPLN. INFO.: US 1994-183119 B2 19940118  
US 1994-312604 B2 19940928  
WO 1995-US829 W 19950118

AB The present invention provides zinc finger nucleotide binding protein variants that have at least two zinc finger modules that bind to a target cellular nucleotide sequence and modulate the transcriptional function of the cellular nucleotide sequence. Also provided are methods of use of such zinc finger nucleotide binding protein variants and methods for isolating the same using expression libraries encoding the protein variants contg. randomized substitutions of amino acids. Exemplary zinc finger nucleotide binding protein variants of the invention include two cysteines and two histidines whereby both cysteines are amino proximal to both histidines. Design and construction of variants of the **zinc finger protein Zif/268** are disclosed. Construction of multifinger proteins utilizing repeats of the first finger of Zif/268 is described.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE

Searcher : Shears 308-4994

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IN THE RE FORMAT

L22 ANSWER 9 OF 13 WPIDS (C) 2003 THOMSON DERWENT  
ACCESSION NUMBER: 2001-308618 [32] WPIDS  
DOC. NO. CPI: C2001-095392  
TITLE: New fusion protein containing nucleotide-binding  
and ligand-binding domains, useful e.g. in gene  
therapy of cancer, provides ligand-activated  
control of gene expression.  
DERWENT CLASS: B04 D16  
INVENTOR(S): BARBAS, C F; BEERLI, R; KADAN, M  
PATENT ASSIGNEE(S): (NOVS) NOVARTIS AG; (SCRI) SCRIPPS RES INST  
COUNTRY COUNT: 94  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001030843	A1	20010503	(200132)*	EN	217
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2001011438	A	20010508	(200149)		
EP 1226168	A1	20020731	(200257)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001030843	A1	WO 2000-EP10430	20001023
AU 2001011438	A	AU 2001-11438	20001023
EP 1226168	A1	EP 2000-972849	20001023
		WO 2000-EP10430	20001023

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001011438	A Based on	WO 200130843
EP 1226168	A1 Based on	WO 200130843

PRIORITY APPLN. INFO: US 2000-586625 20000602; US 1999-433042  
19991025

AN 2001-308618 [32] WPIDS  
AB WO 200130843 A UPAB: 20010611  
NOVELTY - Fusion protein (I) comprising a nucleotide-binding domain  
(NBD) linked to a ligand-binding domain (LBD) of an intracellular  
receptor (ICR). NBD is a polydactyl **zinc finger**  
**protein**, or a modular part of it, that interacts  
specifically with a contiguous sequence of at least 3 nucleotides  
(nt), and (I) functions as a ligand-activated transcriptional  
regulator.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included

fort he following:

- (a) nucleic acid (II) that encodes (I);
- (b) vector containing (II);
- (c) cell containing the vector of (b);
- (d) combination of (I) or (II) with a regulatable expression cassette containing at least one response element recognized by NBD;
- (e) composition for regulating gene expression comprising (I) or (II) plus an excipient;
- (f) regulating gene expression in a cell by introducing (I) or (II) then treating the cell with a ligand that interacts with LBD; and
- (g) non-viral delivery system comprising (I) or (II).

ACTIVITY - Anticancer; Antiproliferative.

MECHANISM OF ACTION - Ligand-activated regulation of transcription.

USE - (I), or the nucleic acid (II) that encodes it, is used to regulate gene expression, particularly in gene therapy, especially of malignant or non-malignant proliferative disease (cancer, psoriasis, Behcet syndrome etc.), e.g. where induced by viruses in humans or plants, also genetic and/or acquired diseases.

ADVANTAGE - (I) can be designed to target any selected gene (endogenous or exogenous), and can be made to have different selectivity or specificity for endogenous or exogenous ligands.  
Dwg.0/27

L22 ANSWER 10 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 2001:249053 BIOSIS  
DOCUMENT NUMBER: PREV200100249053  
TITLE: **Zinc finger protein**  
derivatives and methods therefor.  
AUTHOR(S): **Barbas, Carlos F.**; Gottesfeld, Joel M. (1);  
Wright, Peter E.  
CORPORATE SOURCE: (1) Del Mar, CA USA  
ASSIGNEE: The Scripps Research Institute  
PATENT INFORMATION: US 6140466 October 31, 2000  
SOURCE: Official Gazette of the United States Patent and  
Trademark Office Patents, (Oct. 31, 2000) Vol. 1239,  
No. 5, pp. No Pagination. e-file.  
ISSN: 0098-1133.  
DOCUMENT TYPE: Patent  
LANGUAGE: English

AB **Zinc finger proteins** of the Cys2 His2 type represent a class of malleable DNA binding proteins which may be selected to bind diverse sequences. Typically, **zinc finger proteins** containing three zinc finger domains, like the murine transcription factor Zif268 and the human transcription factor Sp1, bind nine contiguous base pairs (bp). To create a class of proteins which would be generally applicable to target unique sites within complex genomes, the present invention provides a polypeptide linker that fuses two three-finger proteins. Two six-fingered proteins were created and demonstrated to bind 18 contiguous bp of DNA in a sequence specific fashion. Expression of these proteins as fusions to activation or repression domains allows transcription to be specifically up or down modulated within cells. Polydactyl **zinc finger proteins** are broadly applicable as genome-specific transcriptional switches in gene therapy strategies and the development of novel transgenic plants and animals. Such proteins are useful for inhibiting,



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activating or enhancing gene expression from a zinc finger-nucleotide binding motif containing promoter or other transcriptional control element, as well as a structural gene or RNA sequence.

L22 ANSWER 11 OF 13 SCISEARCH COPYRIGHT 2003 ISI (R)  
ACCESSION NUMBER: 1999:237427 SCISEARCH  
THE GENUINE ARTICLE: 177RH  
TITLE: Toward controlling gene expression at will:  
Selection and design of zinc finger domains  
recognizing each of the 5'-GNN-3' DNA target  
sequences  
AUTHOR: Segal D J; Dreier B; Beerli R R; **Barbas C F**  
(Reprint)  
CORPORATE SOURCE: SCRIPPS RES INST, SKAGGS INST CHEM BIOL, BCC-515,  
10550 N TORREY PINES RD, LA JOLLA, CA 92037  
(Reprint); SCRIPPS RES INST, SKAGGS INST CHEM BIOL,  
LA JOLLA, CA 92037; SCRIPPS RES INST, DEPT BIOL MOL,  
LA JOLLA, CA 92037  
COUNTRY OF AUTHOR: USA  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF  
THE UNITED STATES OF AMERICA, (16 MAR 1999) Vol. 96,  
No. 6, pp. 2758-2763.  
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE  
NW, WASHINGTON, DC 20418.  
ISSN: 0027-8424.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 35

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We have taken a comprehensive approach to the generation of novel DNA binding zinc finger domains of defined specificity. Herein **rye** describe the generation and characterization of a family of zinc finger domains developed for the recognition of each of the 16 possible 3-bp DNA binding sites having the sequence 5'-GNN-3'. Phage display libraries of **zinc finger proteins** were created and selected under conditions that favor enrichment of sequence-specific proteins. Zinc finger domains recognizing a number of sequences required refinement by site-directed mutagenesis that was guided by both phage selection data and structural information. In many cases, residues not expected to make base-specific contacts had effects on specificity. A number of these domains demonstrate exquisite specificity and discriminate between sequences that differ by a single base with >100-fold loss in affinity. We conclude that the three helical positions -1, 3, and 6 of a zinc finger domain are insufficient to allow for the fine specificity of the DNA binding domain to be predicted. These domains are functionally modular and may be recombined with one another to create polydactyl proteins capable of binding 18-bp sequences with subnanomolar affinity. The family of zinc finger domains described here is sufficient for the construction of 17 million novel proteins that bind the 5'-(GNN)(6)-3' family of DNA sequences. These materials and methods should allow for the rapid construction of novel gene switches and provide the basis for a universal system for gene control.

L22 ANSWER 12 OF 13 HCAPLUS COPYRIGHT 2003 ACS

Searcher : Shears 308-4994

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ACCESSION NUMBER: 1998:795122 HCAPLUS  
DOCUMENT NUMBER: 130:33969  
TITLE: Design and construction of **zinc finger protein** derivatives  
INVENTOR(S): **Barbas, Carlos F., III**; Gottesfeld, Joel M.; Wright, Peter E.  
PATENT ASSIGNEE(S): The Scripps Research Institute, USA  
SOURCE: PCT Int. Appl., 159 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9854311	A1	19981203	WO 1998-US10801	19980527
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 6140466	A	20001031	US 1997-863813	19970527
AU 9878003	A1	19981230	AU 1998-78003	19980527
EP 988377	A1	20000329	EP 1998-926088	19980527
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002502249	T2	20020122	JP 1999-500870	19980527
PRIORITY APPLN. INFO.:			US 1997-863813	A1 19970527
			WO 1998-US10801	W 19980527

AB **Zinc finger proteins** of the Cys2His2 type represent a class of malleable DNA-binding proteins which may be selected to bind diverse sequences. Typically, **zinc finger proteins** contg. 3 zinc finger domains, like the murine transcription factor Zif268 and the human transcription factor Sp1, bind 9 contiguous base pairs (bp). To create a class of proteins which would be generally applicable to target unique sites within complex genomes, the present invention provides a polypeptide linker (Thr-Gly-Glu-Lys-Pro) that fuses two 3-finger proteins. Two 6-fingered proteins were created and demonstrated to bind 18 contiguous bp of DNA in a sequence-specific fashion. Expression of these proteins as fusions to activation or repression domains (e.g., with Jun/Fos leucine zipper domains, the Kruppel-assocd. box A domain, or the transcriptional activation domain of herpes simplex virus VP16 protein) allows transcription to be specifically up- or down-modulated within cells. Polydactyl **zinc finger proteins** are broadly applicable as genome-specific transcriptional switches in gene therapy strategies and the development of novel transgenic **plants** and animals. Such proteins are useful for inhibiting, activating or enhancing gene expression from a zinc finger-nucleotide binding motif contg. promoter or other transcriptional control element, as well as a structural gene or RNA sequence.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE

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TITLE: Design of polydactyl **zinc-finger proteins** for unique  
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AUTHOR(S): Liu, Qiang; Segal, David J.; Ghiara, Jayant B.;  
**Barbas, Carlos F., III**  
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like the murine transcription factor Zif268 and the human  
transcription factor Sp1, bind nine contiguous base pairs. To  
create a class of proteins that would be generally applicable to  
target unique sites within complex genomes, the authors have  
utilized structure-based modeling to design a polypeptide linker  
that fuses two three-finger proteins. Two six-fingered proteins  
were created and demonstrated to bind 18 contiguous bp of DNA in a  
sequence-specific fashion. Expression of these proteins as fusions  
to activation or repression domains allows transcription to be  
specifically up- or down-modulated within human cells. Polydactyl  
**zinc-finger proteins** should be broadly  
applicable as genome-specific transcriptional switches in gene  
therapy strategies and the development of novel transgenic  
plants and animals.

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